

ASPECTS OF FLIGHT BEHAVIOR IN  
*CALINEURIA CALIFORNICA*  
(PLECOPTERA: PERLIDAE) FROM A ROCKY  
MOUNTAIN LAKE OUTLET SYSTEM<sup>1</sup>

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**ABSTRACT:** Flight frequency of male and female *Calineuria californica* was measured on three 2-day periods during their June-July, 1987, emergence. Light energy, % relative humidity, and temperature were measured to identify relationships with flight periodicity, sites of water contact, and longevity. Peaks in male flight were related to evapotranspiration potential (PET), and female oviposition peaks were related to light intensity. Sexes exhibited significantly different choices in water contact, with males landing mostly at pools and females at riffles. Both sexes lived longer under shaded experimental conditions when water was available. Male and female *Calineuria californica* fly with different periodicities due to different environmental cues.

*Calineuria californica* (Banks) is widespread in western North America (Stark *et al.* 1986), where it is often abundant and contributes substantially to the community dynamics in many stream systems. Siegfried & Knight (1976a) reported aspects of its life history and growth, and confirmed a 2-3 year life cycle (Sheldon 1969, Heiman & Knight 1975). Delayed hatching occurs in some systems (Siegfried & Knight 1978). These studies and Baumann *et al.* (1977) report *C. californica* emergence in June and July.

Little is known of adult flight behavior for *C. californica* or any other species of summer emerging Plecoptera. Some observations are reported by Hynes (1972) and Macan (1974). Roos (1957), Elliott (1967), Shumacher (1970), and Madsen *et al.* (1973) have studied flight direction of Plecoptera and Ephemeroptera in accordance with Muller's (1954) hypothesis of drift recolonization and dispersal by upstream flight, but flight periodicity and behavior in relation to abiotic variables has been poorly studied in these orders. This study had 3 major objectives: 1) to identify relationships between *C. californica* flight and the abiotic variables temperature, relative humidity, and light, 2) to describe any unique flight behavior(s) in both sexes in relation to the abiotic variables, and 3) to experimentally measure longevity in relation to the abiotic variables.

## METHODS

The study site was a 30 m section of Owl Creek, located 1.5 km below mesotrophic Placid Lake (460 ha, elev. = 1255 m) in the Clearwater River

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drainage, Missoula Co., Montana. Oswood (1976a) and Sheldon & Oswood (1977) found *C. californica* to be the dominant stonefly in Owl Creek. The site was 5-12 m wide, and its east-west position and open riparian canopy of tall conifers allowed maximum sunlight exposure. The 30 m segment was divided into equal 15 m riffle and pool sections by wooden stakes and plastic flagging to facilitate flight observations and enumeration.

All measurements were taken on 3 pairs of sampling dates during the emergence period (June 25-26, July 2-3, July 9-10, 1987). Adults were captured with a sweep net attached to an extendable 3.6 m tubular aluminum handle during one 15 min period within each 1/2 h from 16:00-22:00 and 06:00-12:00 the next morning. Numbers of *C. californica* flying were recorded and categorized under sex and water contact (riffle, pool, or transient flight with no contact). Size difference frequently confirmed by net captures, enabled sexing of flying individuals. Percent relative humidity (R.H.) and air temperature ( $^{\circ}\text{C}$ ) were measured with a wet bulb-dry bulb Sling Psychrometer (J.L. Stortz Div., Perkasio, PA.), and light intensity was measured with a model 268WA620 light meter (Kahl Scientific, San Diego, CA.), and converted from Ft. Candles to Lux. All measurements were taken every 30 min during the study periods.

Net-captured stoneflies were placed in 10 cm X 7.5 cm X 5 cm wire screen cages. The top side was covered with rigid aluminum and secured with rubber bands to keep debris or rain from entering. These were kept in a cooler at 3-5 $^{\circ}\text{C}$  and 80-90 % R.H. for no more than 12 h until used for longevity experiments. Mature nymphs were captured with a D-frame kick net, and reared to adults in cylindrical screen wire cages placed in the stream. Reared virgin adults were held in cages under the same conditions as field-collected adults. Exuviae were removed from 7 exposed boulders having an above-water surface area of approximately 4.3-5.1 m<sup>2</sup> to monitor emergence during the 3-week sampling period.

Two locations at the University of Montana Biological Station were used for longevity experiments: (1) 2 m from shore of Yellow Bay, Flathead Lake (elevated 1 m above the water surface and positioned in full sunlight), and (2) along Royce Creek (elevated 20 cm above the stream under a fully shaded canopy). Groups of caged individuals (no more than 4/cage of virgins or field-collected with or without water access, males or females) were kept in each of these locations. For the water access variable, 5 X 0.5 cm petri dishes were placed in all cages, with or without water (replaced daily or as necessary in the water available treatment). Light intensity, R.H. and temperature were recorded every 30 min during the same day-periods as on-site measurements at Owl Creek. Vitality was checked 2-3 times daily, and dead adults were removed and placed in 80% ethanol. Preserved

females were later dissected with forceps under a binocular dissecting microscope to determine presence of remaining eggs.

Data analysis was completed with the North Texas State University computer system (National Advanced Systems 8043 Dual Processor) and the statistical analysis system (SAS). Tests included: 1) simple and multiple regressions with and without data transformations for flight frequency data, 2) analysis of variance (ANOVA) for longevity data including a Student Newman-Keuls (SNK) multiple range test, and 3) a paired T-Test for riffle vs. pool water contact in males and females. Since the validity of multiple regression depends on the absence of colinearity between independent variables, an attempt was made to combine these into one useable variable by computation of Evapotranspiration Potential (PET), since colinearity was expected between R.H., temperature, and light. This parameter (PET) gives the potential rate of air to cause evaporation from a transpiring surface such as a plant, or in this application, a stonefly adult, in mm of water per day from a particular known surface area. We calculated this parameter utilizing the Penman (1956) equation, which is treated in detail in the works of Penman (1955), Oliver (1973), and Eagleman (1976). Conversions from Lux of light to calories of energy were acquired from Weast (1986); these conversions are not exact, but give a relative unit of measure of light energy for purposes of this study. Approximate stonefly surface area was calculated using ocular micrometer measurements of the 3 body regions in both males and females.

## RESULTS AND DISCUSSION

Emergence of *C. californica* was in progress and continued throughout the 3 week study period; number of exuviae removed from rock surfaces totaled 107, 63, 37, and mature nymphs collected for rearing totaled 31, 20, and 5 for the 3 pair of sampling dates, respectively. Assuming both sexes had similar preferences for emergence sites at Owl Creek, collected exuvia and reared nymphs reflected a population of 55% females and 45% males, suggesting a possible protandrous emergence in the early, unmonitored portion of the emergence period. No other large perlids were flying in large numbers; 3 *Hysperoperla pacifica* (Banks) were captured, and could easily be distinguished from *C. californica* based on size and body coloration. The 3 paired study dates were selected to insure a good range of weather conditions and abiotic variables, from warm and clear on June 25 (PM), 26 (AM), July 3 (AM), and July 9 (PM), to cloudy, cooler weather with rain nearby on July 2 (PM) and 10 (AM). Combining data from morning (AM) and evening (PM) flight did not increase the significance of any statistical relationship with abiotic variables, and therefore AM and PM data are reported separately in Table 1. Natural log transformations increased the

data fit and statistical significance of all relationships, and are also reported in Table 1.

During longevity experiments, location 1 with full sunlight had mean temperatures 5% higher, relative humidities 8% lower, and light ca. 300% higher than shaded location 2. Using the Penman (1956) equation, these data convert to a mean PET 93% higher at location 1 during the hours between 06.00-22.00. Approximate mean stonefly surface area was calculated as 4.48 cm<sup>2</sup> for males and 6.95 cm<sup>2</sup> for females.

Male flight per 15 min. period peaked from 16:30-17:30 on June 25 and July 9 (Fig. 1). About 90% of male flight observed was when R.H. was below 40%, temperature was above 20°C, and light was above 10,765 Lux. The highest R<sup>2</sup> of models tested statistically was generated by a multiple regression with log-transformed flight frequency and the 3 abiotic variables for both AM and PM samples. However, due to significant colinearity between R.H., temperature, and light, the most valid statistical relationship was between flight frequency and PET. Evapotranspiration potential accounted for a significant portion of the variation in log-transformed AM flight frequency (R<sup>2</sup> = 0.67) and log-transformed PM flight frequency (R<sup>2</sup> = 0.73, Table 1), and reached a peak at 13.04 mm water/day at 16:30 for males. The largest portion of the variation in flight frequency attributed to any single regression variable was that of temperature in AM samples and % R.H. in PM samples (Table 1). These data suggest that stoneflies, like

Table 1. Summary of significant model statistics for *Calineuria californica* flight frequency in relation to abiotic variables and evapotranspiration potential (PET). All other models tested were not significant.

| Dependent Variable | Independent Variable(s) | Test          | Adjusted R <sup>2</sup> | Prob. {F (α = 0.05)} |
|--------------------|-------------------------|---------------|-------------------------|----------------------|
| ♂ flight (AM)      | temperature             | regression    | 0.51                    | 0.029                |
| ♂ flight (AM)      | R.H.                    | regression    | 0.46                    | 0.042                |
| log ♂ flight (AM)  | temp., R.H., light      | regression    | 0.77                    | 0.431                |
| log ♂ flight (AM)  | PET                     | regression    | 0.67                    | 0.004                |
| ♂ flight (PM)      | temperature             | regression    | 0.59                    | 0.0001               |
| ♂ flight (PM)      | R.H.                    | regression    | 0.67                    | 0.0001               |
| ♂ flight (PM)      | light                   | regression    | 0.66                    | 0.0001               |
| ♂ flight (PM)      | temp., R.H., light      | multiple reg. | 0.75                    | 0.0001               |
| ♂ flight (PM)      | PET                     | regression    | 0.67                    | 0.0001               |
| log ♂ flight (PM)  | temp., R.H., light      | multiple reg. | 0.86                    | 0.0001               |
| log ♂ flight (PM)  | PET                     | regression    | 0.73                    | 0.0001               |
| ♀ flight (PM)      | light                   | regression    | 0.15                    | 0.0196               |
| ♀ flight (PM)      | temp., R.H., light      | multiple reg. | 0.42                    | 0.0007               |
| ♀ flight (PM)      | PET                     | regression    | 0.11                    | 0.0298               |
| log ♀ flight (PM)  | temp., R.H., light      | multiple reg. | 0.56                    | 0.0001               |
| log ♀ flight (PM)  | PET                     | regression    | 0.12                    | 0.021                |

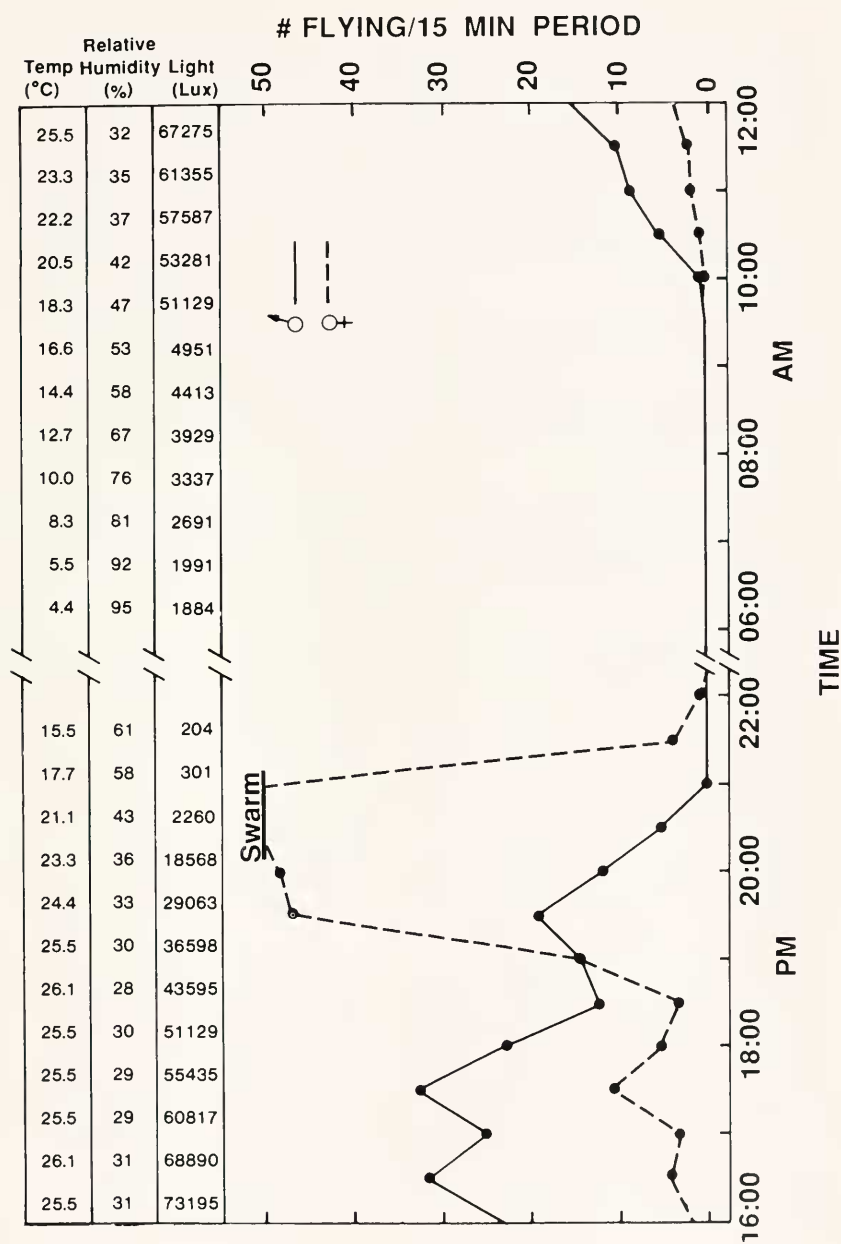


Fig. 1. Flight frequency vs. Time in adult *Calineuria californica* during two, 6 hr periods on June 25-26, 1987 at Owl Creek, Montana. The other sampling periods demonstrated similar flight patterns.



other insects, may require a certain temperature in morning hours to attain heat energy for flight muscles to function, and since 82% of males observed flying made contact with water, late afternoon hours with low % R.H., high temperature, and high PET may stimulate a "drinking" response (dehydrating conditions prevail). During cloudy sampling dates [July 2 (PM), July 10 (AM)], male flight frequency was less, presumably due to higher % R.H. and much lower PET values. Longevity experiments reinforced this presumption, since significantly greater longevity occurred in males that were constantly provided with water (ANOVA,  $N=58$ ,  $R^2=0.88$ ,  $F=56.15$ ,  $P=0.0001$ ). This is consistent with the work of Finni (1975) who suggested adult preference for areas of constant temperature and relatively high humidity, such as under rocks or tree bark. Male *C. californica* at Owl Creek were presumably expending flight energy to increase their longevity through drinking water.

Female flight frequency per 15 min sample peaked between 19:30-21:00 (Fig. 1) during all sets of sampling dates, with swarms of 50+ individuals observed at ca. 20:45. Lower numbers flew during cloudy and cooler weather on July 2 (PM) and this was apparently not due to a reduction in number of adults present, because a 20:45 peak on July 9 with high numbers nearly equalling that of June 25 was observed. About 90% of female flight was observed above 35% R.H., above 18°C, and below 37,675 Lux. The highest  $R^2$  of models tested statistically was generated by a multiple regression with log transformed flight frequency and the 3 abiotic variables for both AM and PM samples. Female flight does not appear to be related to dehydration as is the case with males; % R.H. did not contribute significantly to any model tested, PET did not have a significant relationship with AM flight frequency, and low  $R^2$  were generated with log-transformed PM flight data ( $R^2=0.12$ , Table 1). PET values reached a peak of 19.03 mm water/day at 16:30, but very few females were observed flying near this time period. Larger amounts of fat, and therefore greater water storage in females may make "drinking" behavior less frequent, or females may combine this behavior with their water contact during oviposition to conserve energy. The largest portion of the variation in flight frequency attributed to any single abiotic variable was with temperature in AM samples and light in PM samples. Again, temperature appears necessary for generating flight energy during AM hours, and light is a significant stimulant to female flight in evening hours (Table 1). No oviposition swarm occurred during cloudy conditions on July 2 (PM), even though temperatures were warm enough for flight. About 89% of enumerated females made water contact, and net collected females frequently had attached egg masses on their abdomens. During flight from the canopy, wing beat ceased as far as 10 m above the stream. Females glided to the surface, spent 5-10 s in contact with water to oviposit, then presumed flight to return to the

canopy. This gliding, "dive-bombing" method of oviposition is similar to that reported for *Clioperla clio* (Minshall & Minshall 1966).

Observations of female swarms on June 25 (PM) and July 9 (PM) confirmed light and temperature as plausible cues for stimulating flight. After oviposition and water contact ceased, the swarm gradually moved higher in elevation until 21:15 when the lowest females flying were at tree-top level. This corresponds with the level where direct sunlight still illuminated the tree tops of the riparian canopy, and when temperatures exhibited their most rapid decrease. Convective heat loss and the rise of moist air masses from the stream bed may also have contributed to the rise in the female swarm at dusk.

Female longevity data were similar to males, with significantly greater longevity for those given water and/or maintained in a cooler, shaded, moderately humid environment (Table 2). Since spent females were not expected to live long enough for the difference in abiotic factors to affect them, they were treated as separate groups in the analysis of variance,

Table 2. Summary of longevity statistics for *Calineuria californica*. Analysis of Variance (ANOVA) yielded an  $R^2 = 0.88$ , prob.  $< F = 0.0001$  for males and  $R^2 = 0.82$ , prob.  $> F = 0.0001$  for females, showing a significant difference in mean longevity for both sexes.

| $\sigma$<br>H <sub>2</sub> | location | Collection      | N  | X<br>longevity<br>(hr) | SNK grouping (means with<br>the same letter are not<br>significantly different) |   |   |   |   |
|----------------------------|----------|-----------------|----|------------------------|---------------------------------------------------------------------------------|---|---|---|---|
| yes                        | shade    | reared virgins  | 4  | 243                    | A                                                                               |   |   |   |   |
| yes                        | sun      | reared virgins  | 6  | 138                    |                                                                                 | B |   |   |   |
| no                         | shade    | reared virgins  | 3  | 112                    |                                                                                 | B | C |   |   |
| yes                        | shade    | field collected | 8  | 99                     |                                                                                 |   | C |   |   |
| yes                        | sun      | field collected | 8  | 64                     |                                                                                 |   |   | D |   |
| no                         | shade    | field collected | 15 | 48                     |                                                                                 |   |   | D | E |
| no                         | sun      | reared virgins  | 4  | 48                     |                                                                                 |   |   | D | E |
| no                         | sun      | field collected | 10 | 23                     |                                                                                 |   |   |   | E |
| $\varphi$                  |          |                 |    |                        |                                                                                 |   |   |   |   |
| yes                        | shade    | reared virgins  | 4  | 213                    | A                                                                               |   |   |   |   |
| yes                        | sun      | reared virgins  | 5  | 153                    |                                                                                 | B |   |   |   |
| no                         | shade    | reared virgins  | 3  | 100                    |                                                                                 |   | C |   |   |
| yes                        | shade    | field collected | 16 | 92                     |                                                                                 |   | C |   |   |
| yes                        | sun      | field collected | 15 | 69                     |                                                                                 |   | C | D |   |
| no                         | sun      | reared virgins  | 4  | 60                     |                                                                                 |   | C | D | E |
| no                         | shade    | field collected | 10 | 47                     |                                                                                 |   |   | D | E |
| no                         | shade    | field collected | 10 | 35                     |                                                                                 |   |   | D | E |
| yes                        | shade    | field - spent   | 2  | 30                     |                                                                                 |   |   | D | E |
| yes                        | sun      | field - spent   | 11 | 19                     |                                                                                 |   |   |   | E |
| no                         | shade    | field - spent   | 2  | 18                     |                                                                                 |   |   |   | E |
| no                         | sun      | field - spent   | 7  | 14                     |                                                                                 |   |   |   | E |

which showed significant differences in mean hours kept alive ( $N=67$ ,  $R^2=0.82$ ,  $F=33.77$ ,  $P=0.0001$ ). Longevity summary statistics for both sexes, including Student Newman-Keuls multiple range test, are given in Table 2 and are consistent with the work of Finni (1975) who found that *Allocaenia granulata* (Claassen) given water had significantly greater longevity than those without water. Reared virgin males given water had the greatest longevity ( $N=4$ ,  $\bar{X}=213$  h), and as expected, field collected spent females had the shortest longevity ( $N=7$ ,  $\bar{X}=14$  h).

Difference in male and female flight periodicity was indicated by the 2-3 hr difference in PM flight peaks (Fig. 1). The sexes also exhibited different water-contact sites, with significantly more males landing in the pool segment of the 30 m study site ( $N=320$ ,  $\bar{X}=63.5\%$ , paired  $T=6.01$ ,  $P=0.0001$ ) and 90.8% of females ovipositing in the riffle segment ( $N=374$ , paired  $T=-4.54$ ,  $P=0.0001$ ). Similarly, Macan (1974) postulated that flying insects are attracted to reflective surfaces such as riffles, and ovipositing females during swarms may have the ability to detect the greater range of wavelengths and incident angles of reflected light from a riffle area. This reflected light is greater during clear weather, which may partially explain female oviposition swarms of smaller numbers during cloudy periods such as that observed on July 2 (PM).

## CONCLUSION

It is apparent that flight frequencies in male and female *Calineuria californica* from Owl Creek, Montana, were due to different cues, and occur at different time periods for different reasons due to differences in moisture, light, and temperature. Males appeared to fly in response to dehydration conditions such as low R.H., high temperature and greater sunlight energy, leading to greater PET values. Females responded significantly to light, and had a peak flight period 2-3 hr later than males. Relationships between male flight and % R.H. were stronger than that of females in our study. During morning periods, the effect of temperature thresholds prevailed in keeping stoneflies from flying until sufficient energy was generated to sustain flight later in the day. Longevity data reinforced our presumptions that water was important for extended survival of both sexes, and spent females may be ready to die regardless of experimental conditions. More behavioral experiments with stonefly flight are needed over longer periods of time with other species to better define flight patterns and behavior in Plecoptera.

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